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RIBOFLAVIN IN HUMAN NUTRITION

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RIBOFLAVIN IN HUMAN NUTRITION

Recent studies indicate that riboflavin deficiency is the most prevalent of the uncomplicated avitaminoses and that it ranks second only to calcium as the nutrient in which the American diet is most seriously lacking. In terms of practical dietetics these facts indicate the necessity for increasing the consumption of the important food sources of this vitamin, such as liver and kidney, eggs, milk and other dairy products.

Beginning in 1938, the first studies were made with human subjects to determine the exact nature and scope of the pathological changes incurred by riboflavin deficiency. In addition to the general symptoms of the vitamin deficiency—fatigue, nervousness, poor appetite and digestive disturbances—specific lesions of the eyes, mouth and skin were noted. These disturbances constitute the clinical picture of what is now recognized as a specific disease entity to which has been given the name, ariboflavinosis.

Paralleling progress in the clinical investigation of ariboflavinosis has been the detailed series of studies on human beings designed to determine the actual riboflavin requirements for various age and sex groups. These experiments have brought forth interesting data on the lowest levels of riboflavin intake which can serve to maintain an individual free of any detectable manifestations of riboflavin deficiency. There is no doubt that these painstaking studies have contributed valuable data to the science of human nutrition. In evaluating these data, it must be kept in mind, however, that there is a vast difference between the establishment of a scientific minimum necessary to prevent obvious deficiency symptoms and of an optimum providing incalculable benefits in increased health, vitality and longevity.

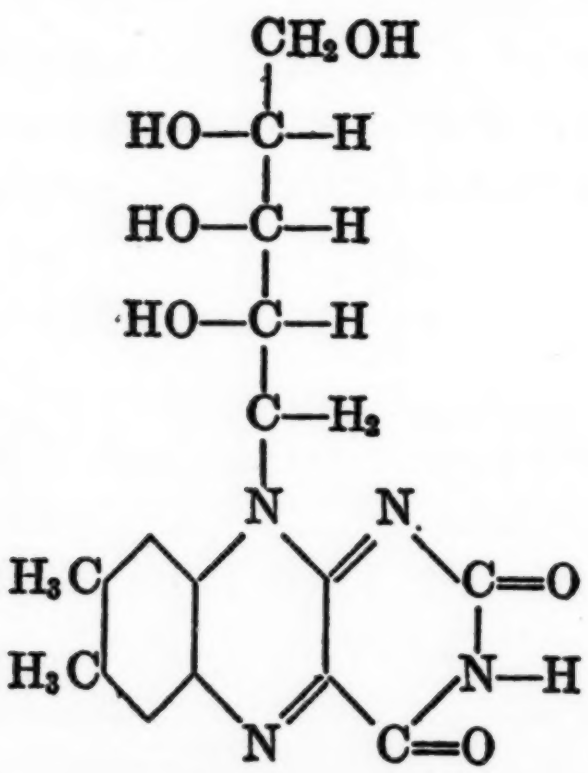
The Discovery of Riboflavin

In 1879, long before its vitamin properties were recognized, riboflavin was discovered by an observant chemist as a fluorescent greenish-yellow pigment in milk to which was given the name lactochrome (1). No more was reported about this coloring material until 1925 when two investigators described it in detail and changed the name from lactochrome to lactoflavin, thereby indicating its chemical relation to the flavin pigments (2).

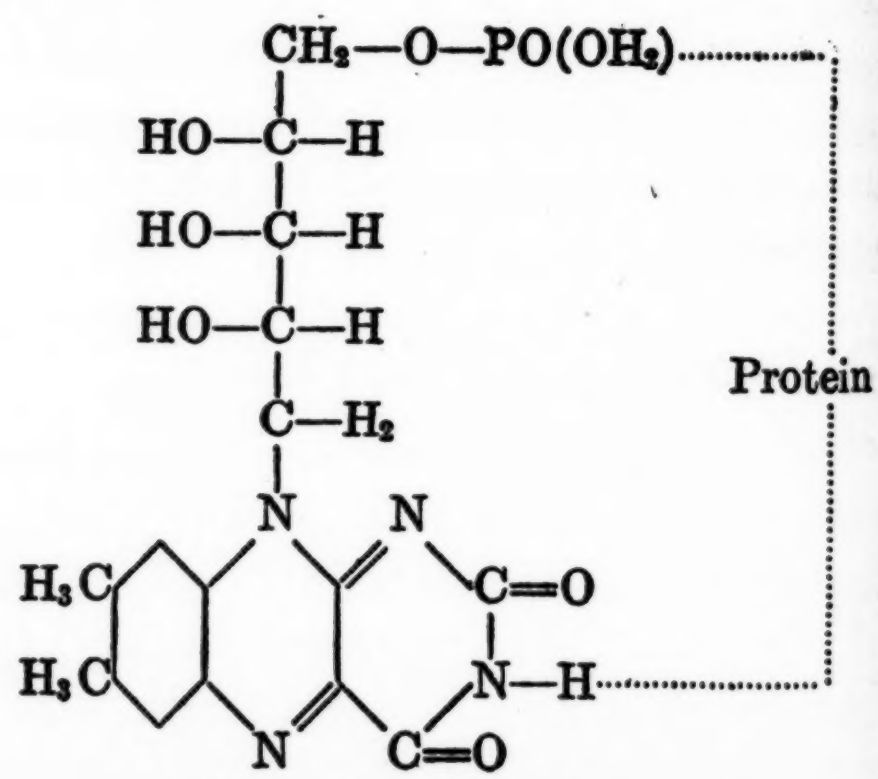
It was not until 1932, however, that the first of a significant series of events occurred providing the initial clue to the biological impor-

tance of lactoflavin. At that time, Warburg and Christian (3) an nounced the discovery of a new oxidation enzyme derived from yeas Because of its yellow color and greenish fluorescence in water, the called the new material "the yellow oxidation enzyme." The follow ing year a second significant discovery was made almost simultaneousl by Lela Booher (4) in Sherman's laboratory at Columbia Universit and by Kuhn, György and Wagner-Jauregg (5) in Europe. The found that a yellow pigment isolated from milk whey or egg wa capable of acting as a growth stimulating supplement to the standar Bourquin-Sherman experimental diet and that, further, the potenc of the material varied directly with the intensity of pigmentation. A comparison of their properties made it apparent that the growth promoting yellow pigment of milk and the yellow oxidation enzym of Warburg and Christian were in some way related.

Investigations as to the chemical nature of the flavins led ulti mately to the isolation and synthesis of the active group. Originall designated by prefixes denoting their source as, lactoflavin (milk), ovoflavin (egg) or hepatoflavin (liver), it was finally recognized tha all were chemically identical. Because of the characteristic ribos sugar grouping common to all, the source names were abandoned and the name *riboflavin* was officially adopted for the newly-recog nized growth material. Subsequently, it was shown that riboflavin is the prosthetic or active portion of the "yellow oxidation enzyme," the remainder being a complex protein molecule to which the ribo flavin is bound by means of a phosphoric acid group.



Riboflavin



Warburg's Yellow Enzyme

While the chemical investigations of riboflavin and the yellow enzyme were progressing, active research was being conducted on

the biological significance of riboflavin. Results of diverse investigations were rapidly converging to reveal the identity of riboflavin and the animal nutrition factor then known as vitamin G.

Studies on vitamin G had their beginnings during the experiments of Goldberger seeking the anti-pellagra factor.* In 1926, he had differentiated the "water-soluble vitamin B" into a complex containing at least two entities, one the heat-labile anti-beriberi factor (vitamin B_1) and the other, a heat stable component (vitamin B_2) thought to be a single "anti-pellagra factor". Not until some years later was the multiple nature of "vitamin B_2" elucidated and the anti-pellagra component, now known as nicotinic acid, differentiated from the growth factor, vitamin G. It was not long before the chemical properties of vitamin G and those of riboflavin were perceived to be closely similar and the final recognition of the pigment, riboflavin, as a vitamin resulted.

Significance of Riboflavin in Human Nutrition

Ariboflavinosis: Although a vast literature dealing with the importance of riboflavin in the nutrition of rats, dogs, pigs, chicks and other animals accumulated rapidly following the increased knowledge concerning riboflavin, hardly anything was known about the significance of this vitamin in human nutrition until six years ago. With the availability of pure nicotinic acid and pure riboflavin and with the identification of nicotinic acid as the pellagra-preventive substance, it became possible to separate the symptoms of riboflavin deficiency from those of nicotinic acid deficiency in the complex picture of pellagra. In 1938 Sebrell and Butler (6) published a preliminary report on the first studies conducted with human beings to determine the effects of riboflavin deficiency. They found that the atypical pellagrous symptoms previously described by Goldberger as "pellagra sine pellagra" (pellagra without the characteristic skin lesions) were due to riboflavin deficiency and not to nicotinic acid deficiency. This confirmed the multiple deficiency nature of pellagra.

The changes in human riboflavin deficiency as observed by Sebrell and Butler (6, 7) consisted of lesions on the lips which began with an unusual paleness of the mucosa in the angles of the mouth. This pallor was soon followed by maceration and the appearance of small fissures which eventually became encrusted and scaly. At the same time the lips became abnormally red, shiny and superficially denuded

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* See *Borden's Review of Nutrition Research*, October, November, 1943.

along the line of closure. These lesions of the lips were named "cheilosis." Lesions about the nose, ears and eyelids were noted in addition to the cheilosis. Sebrell and Butler reported that all of the lesions occurred on a diet deficient in riboflavin, disappeared with the administration of riboflavin and recurred when the vitamin was withdrawn. Nicotinic acid had no effect on the riboflavin deficiency lesions. Accordingly, these lesions were classed as specific evidence of riboflavin deficiency and the entire clinical syndrome was given the name "ariboflavinosis."

Closely following the work of Sebrell and Butler, beneficial results with riboflavin in the treatment of pellagra were reported by Vilter, Vilter and Spies (8). Oden and co-workers (9), Spies, Bean and Ashe (10) and others reported the detection and successful treatment of clinical cases of ariboflavinosis and its successful treatment with riboflavin. As studies of human riboflavin deficiency increased in scope, new manifestations of ariboflavinosis became apparent. Spies *et al* (11) described eye lesions characterized by conjunctivitis, tearing and burning of the eyes and failing vision occurring in conjunction with other symptoms of ariboflavinosis, all of which responded to riboflavin therapy. Jolliffe *et al* (12) reported recognition of ariboflavinosis in patients in New York hospitals and observed additional skin lesions of a seborrheic nature, while Sydenstricker and co-workers (13) reported the incidence of ariboflavinosis in the South. By 1940, the symptom of glossitis (inflammation of the tongue) was added to the picture of ariboflavinosis by Kruse and associates (14, 15) who also explained the occurrence of ocular lesions as being due to interstitial keratitis.

Vascularization of the cornea in human beings—a condition in which congested blood vessels cover the corneal area of the eye—was first suggested as a symptom of riboflavin deficiency by Kruse *et al* (14) on the basis of original animal studies by Bessey and Wolbach (16) in which it had been demonstrated that rats fed a riboflavin-deficient diet developed evidence of corneal vascularization. Kruse and co-workers postulated further that symptoms of tiredness and burning of the eyes, sandy sensation under the lids and tearing could also be manifestations of riboflavin deficiency.

The subject of corneal vascularization and eye fatigue has recently received particular attention in connection with war aviation problems in the Royal Canadian Air Force. Last year a study of these symptoms as they occurred in R.C.A.F. personnel was undertaken by Tisdall, McCreary and Pearce (17). They found a relatively

large incidence of ocular disturbances, particularly after flights in bright weather when the riboflavin content of the eyes had been exposed to the destructive effects of light. Riboflavin therapy in large dosages resulted in marked cessation of symptoms in from 70 to 95 per cent of the men. No improvement in vascularity was noted among the controls, although symptoms of eye fatigue disappeared spontaneously in about 10 percent of the cases. According to Tisdall *et al,* "with R.C.A.F. personnel in areas where milk, the best source of riboflavin in the diet, was not available, prevalence and severity of corneal vascularization was markedly increased."

From the point of view of distress and disability, the eye lesions are the most important symptoms of riboflavin deficiency. They do not appear to be present invariably in ariboflavinosis, however, nor can all cases of ocular keratitis be attributed to lack of riboflavin. The usual symptoms appearing in the clinical picture of ariboflavinosis have been summarized as follows by Sebrell (18):

> "(1) *Ocular lesions,* consisting usually of a vascularizing keratitis with photophobia, dimness of vision, severe injection of the vessels of the fornix and sclera, burning of the eyes, lacrimation, and, in severe cases, opacities of the cornea; (2) *oral lesions,* consisting usually of linear fissures in the angles of the mouth, a reddened, shiny, denuded appearance of the lower lip, and a flattening of the papillae of the tongue, which becomes magenta red in color; (3) *dermal lesions,* consisting usually of seborrheic accumulations in the folds of the skin, especially in the nasolabial folds, around the eyelids, on the ears, and in some cases comedones and a sharkskin-appearing lesion on the nose and over the malar eminences. In some cases the seborrheic dermal lesions may be extensive and may involve other regions of the body."

Anemia: During the past few years reports in the scientific literature have appeared suggesting a possible relationship between riboflavin deficiency and anemia in experimental animals. For some time it appeared likely that riboflavin might be identical with the "extrinsic factor" of Castle, an unidentified factor in food which, in combination with a factor in gastric secretions (the so-called "intrinsic factor") produces the anti-pernicious anemia factor. Experimental evidence, however, has not confirmed this supposition at the present time. That riboflavin plays a role in the regeneration of blood following experimentally induced anemia, however, has been demonstrated by several workers. György, Robscheit-Robbins and Whipple (19) reported definite increase in hemoglobin formation in anemic dogs fed riboflavin. More recently, Spector *et al* (20) confirmed this work. Whether these experimental results apply to human beings is still a matter of conjecture and introduces important new considerations in the problem of nutritional anemia.

Biological Action of Riboflavin: Riboflavin occurs in foods generally in combined form as riboflavin phosphate, although some foods, such as cow's milk, contain the free form. Free riboflavin must be combined with phosphoric acid in the body before it can be utilized. Following phosphorylation, the phosphoric ester of riboflavin combines with a protein to form Warburg's yellow enzyme. This enzyme forms part of a complex system which is essential for intra-cellular respiration of all body cells and for the regulation of carbohydrate and fat metabolism. In areas devoid of blood supply, such as the cornea, it is believed that this enzyme system is the only means whereby vital oxidative functions are carried out. Various derivatives of riboflavin combine with specific proteins to form other important enzymes, but not much is known concerning their significance. Spector *et al* (20) have conjectured that riboflavin may be concerned in the metabolism and arrangement of the amino acids of the protein in the hemoglobin molecule through one of its enzyme systems which influences amino acid metabolism. The relationship between the biological action of riboflavin and the recognized clinical manifestations of riboflavin deficiency, however, still remains obscure.

Estimation of Human Requirement

A common method for estimating human vitamin requirements involves studies of the concentration of the vitamin in the blood or measurement of the amount excreted in the urine over a 24-hour period. In the case of riboflavin, the urinary excretion method has been used predominantly because analytical procedures for the detection of flavins in the urine have been perfected to a greater degree than have those for detection of the vitamin in the blood.

Principles of Urinary Excretion Method: This method is based upon the observation that a normal adult on an average diet customarily excretes a fairly constant amount of riboflavin during any given 24-hour period. This value varies within a somewhat wide range for different people, depending upon body weight, daily intake and individual differences in absorption, utilization and state of tissue saturation. For a given individual, however, on a diet more or less the same from day to day, the amount excreted remains almost consistently the same. Under certain conditions this constant excretion value can be changed. For example, riboflavin intakes considerably above the usual daily consumption result in an increased excretion level and likewise, unusually low intakes result in a marked drop in the excretion of the vitamin. From these observations, it seemed

logical to conclude that the amount of riboflavin in the diet necessary to maintain a constant excretion level within normal ranges indicates the probable requirement of the individual.

Excretion Studies: Among the earliest studies were those of Emmerie (21) in 1936. He found normal adult males on an average diet excrete from 819 to 1250 micrograms of riboflavin per day and that unusually high intakes are reflected in an increased excretion level. He concluded that the increased excretion occurs when the tissues are "saturated" with the vitamin. Applying these observations to an experiment with two young men, he reported the following data:

	Subject A	*Subject B*
Average normal excretion on unrestricted diet	952 mcgm.	885 mcgm.
Extra riboflavin*	5710 mcgm.	4240 mcgm.
Riboflavin excreted	3283 mcgm.	1797 mcgm.

* Extra amounts of riboflavin ingested in the form of liver.

The probable daily requirement of these individuals can be calculated from these data. Subject A, for example, excreted normally an average daily amount of 952 mcgm. of riboflavin. This is within the normal range of 819 to 1250 previously established and since the subject was free of any symptoms of vitamin deficiency, the diet was apparently providing at least the minimal amount of riboflavin. With the sudden enrichment of the diet by a large amount of riboflavin-rich food, bringing the intake up to 5710 mcgm. of riboflavin, the amount of the vitamin excreted rose sharply to 3283 mcgm. Thus, on a diet providing 5710 mcgm. of riboflavin, Subject A apparently had enough of the vitamin to satisfy body requirements, to maintain the average excretion level (952 mcgm.) and, in addition, 2331 mcgm. to spare. It may be concluded, then, that without the extra 2331 mcgm., Subject A would be receiving just the right amount to maintain minimum requirements. Subtracting 2331 from 5710 (the total intake) would indicate this amount to be 3379 mcgm. or about 3.3 mg. On the same basis, the requirement for Subject B would also approximate 3.3 mg. of riboflavin per day. It should be pointed out that these figures are not precise values inasmuch as in this type of experiment all of the extra amounts of the vitamin ingested may not appear in the urine. Some of the excess may be destroyed, or excreted in small, fairly constant amounts in the feces; some may be retained as a stored reserve, or the tissues may even develop a higher tolerance or saturation threshold for the vitamin.

Additional observations on the average daily excretions of riboflavin for normal males were recorded by Ferrebee (22). The ranges

were from 700 to 1700 mcgm. per day which agrees fairly well with Emmerie's 819 to 1250 mcgm., taking into account the differences in body weights, dietary differences and other factors.

In the meantime, other workers engaged in the study of human riboflavin excretions, reported interesting data with regard to females. In 1941, Strong, Feeney, Moore and Parsons (23) recorded excretion values for normal individuals on unrestricted diets. From their data an average range of values for riboflavin excreted by women would appear to be from 500 to 800 mcgm. per day. Having tentatively established a range of excretion values for females on the basis of their limited studies, Parsons and co-workers attempted to correlate intake-output relations with the requirement for the vitamin. They reported the following data as a result of tests on four college women ingesting a controlled diet of known riboflavin content.

24-Hour Urinary Excretion of Riboflavin on Controlled Diets

Days	Subject 3		Subject 4		Subject 5		Subject 6	
	Intake	Excretion	Intake	Excretion	Intake	Excretion	Intake	Excretion
	mcgm.	mcgm.	mcgm.	mcgm.	mcgm.	mcgm.	mcgm.	mcgm.
1, 2	1653	560	1653	320	1464	320	1464	380
3, 4	1653	460	1653	300	1464	360	1464	320
5, 6	1653	290	1653	150	1464	240	1464	270
7, 8	1130	146	1258	94	1258	76	1125	196
9, 10	1130	102	1258	98	1258	78	1125	132
11, 12	1130	74	1258	56	1258	54	1125	76
13, 14	1927	118	1938	92	1869	104		
15	1927	120	1938	154	1869			
16*	3927	560	3938	440	3869	380		
17†	6927	3780	6938	3280				
18	1927	560	1938	470				

* 2 mg. of riboflavin were administered orally at 9.00 a.m.
† 5 mg. of riboflavin were administered orally at 6.00 p.m.

A number of interesting conclusions may be drawn from these data*.

*For example, the average normal excretion of Subject 3 had been previously estimated to be approximately 650 mcgm. per day. On an intake of 1653 mcgm. of riboflavin this value dropped to 560 on the first day and continued dropping until a level of 290 was reached after a period of six days. This indicated that for Subject 3, a daily intake of 1.6 mg. (i.e. 1653 mcgm.) was insufficient. A further decrease to 1130 mcgm. riboflavin per day resulted in a sharp drop of the urinary output from 290 to 146 mcgm. within two days, with a slow continued drop until only 74 mcgm. were excreted as compared to the normal amount of 650 mcgm.

Following this period of depletion, the riboflavin intake of Subject 3 was increased from 1330 to 1927 mcgm. per day for three days and the urinary excretion rose slowly from 74 to 120 mcgm. Although 1927 mcgm. represents an intake higher than the original 1653, the initial urinary excretion on this higher intake was lower than it was on the original intake of only 1653 mcgm. This may be interpreted as indicating an attempt of the body to replenish some of its depleted stores demonstrating that following a period of riboflavin privation, the requirement for the vitamin is increased until equilibrium conditions are restored.

After three days on an increased intake of riboflavin, permitting the body to approach its normal level of riboflavin saturation after the depletion period, Subject 3 was given a 2 mg.

supplement of riboflavin orally, bringing the total intake to 3927 mcgm. This resulted in an excretion of 560 mcgm. which is the same as was observed on the 1653 mcgm. intake at the beginning of the experiment indicating that although the body was responding to the higher intake, it had not yet reached saturation. A further supplement of 5 mg. on the next day brought the total intake up to 6927 mcgm. and resulted in a heightened excretion of 3780. This excretion of 3780 mcgm. represents an excess of 3130 mcgm. over the average normal excretion of 650 mcgm. Subtracting this 3130 mcgm. excess from the 6927 mcgm. intake, leaves 3797 mcgm. as the amount of riboflavin apparently required to maintain the normal excretion level of 650 mcgm.

In view of the fact that the subject had undergone severe depletion of riboflavin prior to this, the figure of 3797 mcgm. (or 3.8 mg.) was probably higher than the subject's normal requirement. When the riboflavin intake was reduced the following day from 6927 mcgm. to 1927 mcgm., a drop of 5 milligrams, the excretion dropped to 560 mcgm., a value slightly below the average normal excretion value. This would indicate that 1.9 mg. of riboflavin per day was insufficient for Subject 3. Accordingly, a figure somewhere above 1.9 mg. and below 3.8 mg. would represent her probable daily requirement.

When the riboflavin intake was reduced to a level of 1 to 2 mg. per day, the urinary excretions dropped from the normal range for adults (500 to 800 mcgm.) to from 50 to 150 mcgm. This would suggest that 1 to 2 mg. of riboflavin was insufficient for these subjects. On the basis of the data recorded, it might be assumed that a suitable daily intake for females in this group would be somewhere between 2 and 3 mg. per day.

While the studies of Parsons and her co-workers were under way, experiments along the same lines were being conducted on a long-term scale by Sebrell and associates (24) in the United States Public Health Service. These investigators found that women on an institution diet excreted an average of 357 mcgm. riboflavin per day. This value is somewhat lower than the values derived from the data of Parsons *et al,* but this is probably due to the more restricted nature of the institution diet. When ten women were maintained on a highly restricted diet averaging only 0.5 mg. riboflavin per day, the average excretion of riboflavin dropped to 77 mcgm. which was considerably below both the values for the women on an institution diet and for the college women. The ten women on the restricted riboflavin intake were then given varying amounts of riboflavin and the urinary excretion again determined in an attempt to arrive at an estimate of the daily requirement. The data obtained are summarized in the table on page 10.

On an intake of 0.035 milligram riboflavin per kilogram of body weight (1.75 mg. for a 50 kg. woman) the excretions averaged about 140 to 180 mcgm. per day which is far below the averages of Parsons' subjects or those on the institution diet. When the intake reached a level of 0.06 milligram per kilogram of body weight (3.0 mg. for a 50 kg. woman) the average excretions ranged from 793 to 1265 mcgm. per day which is higher than the average normal excretion values for women previously reported. Consequently the require-

Riboflavin intake and excretion

Subject No.	Weight	Period, days	Average riboflavin intake in diet	Supplement of riboflavin, per day	Total riboflavin intake	Average riboflavin excreted in urine in 24 hours	Riboflavin unaccounted for in urine	Riboflavin not accounted for per kilo body weight
	Kilograms		*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
3	47.4	232-252	430	3,640	4,070	2,151	1,919	40.5
8	61.3	220-227	525	2,830	3,355	1,210	2,145	35
8	61.3	250-288	525	4,500	5,125	2,779	2,246	37
10	56.3	220-240	494	2,750	3,244	793	2,451	43.5
11	59.5	280-292	465	2,950	3,415	975	2,440	41
11	59.5	293-305	465	4,600	5,065	2,424	2,641	44.5
39	49.2	245-252	420	3,610	4,030	1,410	2,620	53.0
46	42.8	189-214	433	2,200	2,633	1,265	1,368	32
46	42.8	271-280	358	3,300	3,658	2,433	1,225	28
59	48.6	252-284	556	3,470	4,026	1,848	2,178	45
65	47	250-281	530	3,510	4,040	2,179	1,861	40

ment value, according to Sebrell's data, lies somewhere between 0.035 and 0.06 mg. per kilogram, or about 2.45 mg. per day for the average 50 kg. woman. On this basis, the requirement of the average 70 kg. man would be somewhat more than 3 mg. per day which accords closely with the original estimations of Emmerie.

Need for Additional Studies: From the limited amount of data supplied by the foregoing experiments it would seem that there is fairly general agreement that the requirement for an average adult male is about 3 milligrams of riboflavin per day and a correspondingly smaller amount for females according to weight. It has been suggested, however, that this may represent an amount of riboflavin necessary for saturation rather than a maintenance requirement. In view of the fact that many of these tests were conducted on subjects depleted of their riboflavin stores it may very well be that the requirement values are too high because depleted subjects would have a higher requirement than normal, or at least, average subjects.

The widespread occurrence of ariboflavinosis in the United States, however, coupled with the evidence of these balance studies, would seem to indicate that these estimated requirements, if they are too high, cannot be very much above the actual daily requirement. In further support of setting a liberal requirement for riboflavin are the highly significant studies of Sherman and Ellis (25, 26) with experimental animals. They found that when parallel groups of rats were fed at graded levels of riboflavin intake, beginning with one which supported normal growth and apparently good health, succes-

sively more liberal levels resulted in more rapid growth, earlier maturity, better vigor and reproductive capacity, greater freedom from disease at all ages, and an increased life span characterized by a longer "prime of life" and delayed senility.

In setting dietary standards or allowances consideration must be given, in addition to other factors, to the ability to attain optimum levels by means of ordinary diet. In the case of riboflavin Sebrell (18) has stated that only by the inclusion of liberal quantities of milk in the diet can the high goal of three milligrams or more of riboflavin be obtained. Ingrained food habits and present day food curtailment are but two factors which may combine to render this goal beyond reach. Consequently, while it would seem highly desirable to set a goal of 3 mg. per day, nutritionists are now concentrating their efforts towards the determination of how much less than this amount would still suffice to provide a quantity of riboflavin sufficiently above bare minimum requirements to provide for good maintenance.

The results of these newer studies on the human requirement for riboflavin together with the recommendations of the National Research Council will be presented in the October, 1944 issue of *Borden's Review of Nutrition Research.*

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